# Induction of Post-translational Modifications in Tumour and their Recognition by T cells

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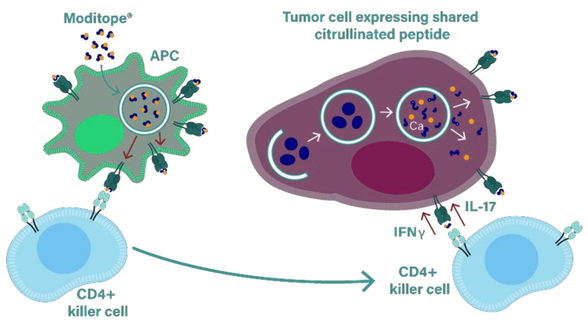
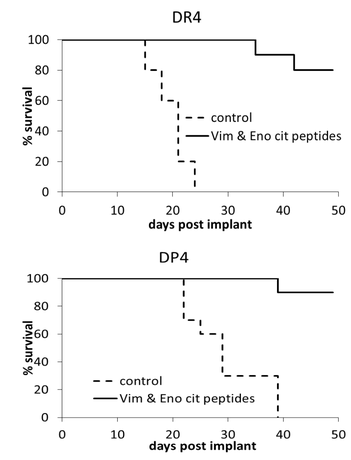
## INTRODUCTION




- **Autophagy is the cells natural protective mechanism against cellular stresses such as nutrient deprivation.**
- **Citrullination occurs during autophagy as a result of high levels of calcium which activate PAD enzymes.**
- **PAD enzymes catalyse the conversion of arginine within a polypeptide to citrulline.**
- **Citrullinated peptides can be presented by MHC Class II on the tumour cell surface.**
- **Immunisation with citrullinated peptides allows CD4 T cell recognition of tumour cells resulting in potent anti-tumour responses.**




## Interferon Gamma induces MHC Class II expression in human cancer cell lines

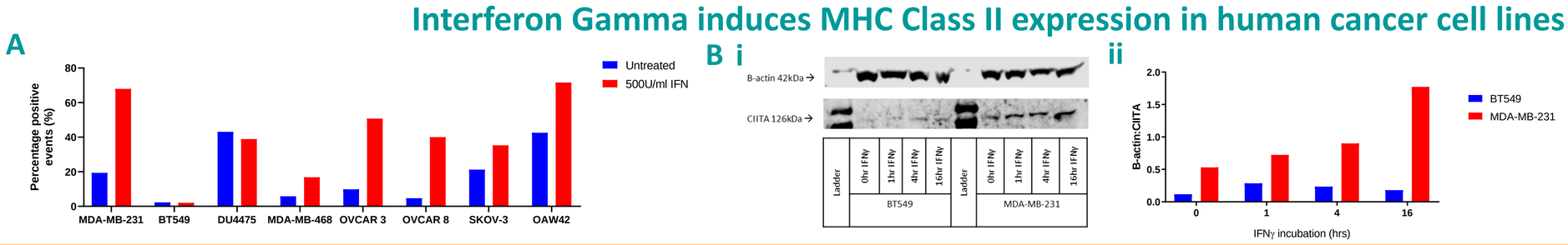



**Figure 1.** Expression of cell surface MHC class II on the triple negative breast cancer cell lines [TNBC] (MDA-MB-231, BT549, DU4475 and MDA-MB-468) and the ovarian cancer cell lines (OVCAR 3, OVCAR 8, SKOV-3 and OAW42). IFNγ increased MHC class II expression in all ovarian cell lines and two TNBC cell lines (a). Successful induction of MHC class II transcriptional activator (CIITA) in MDA-MB-231 but not BT549 incubated with 1000U/ml IFNγ for 0, 1, 4 or 16 hours, correlate with cell surface MHC class II levels (bi). CIIITA signal normalised against β-actin (bii).

## Nutrient starvation and Interferon Gamma treatment upregulates intracellular citrullinated vimentin but also apoptosis

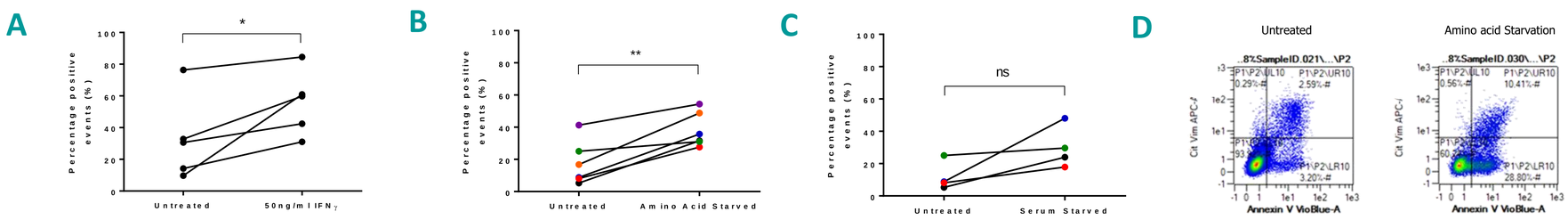
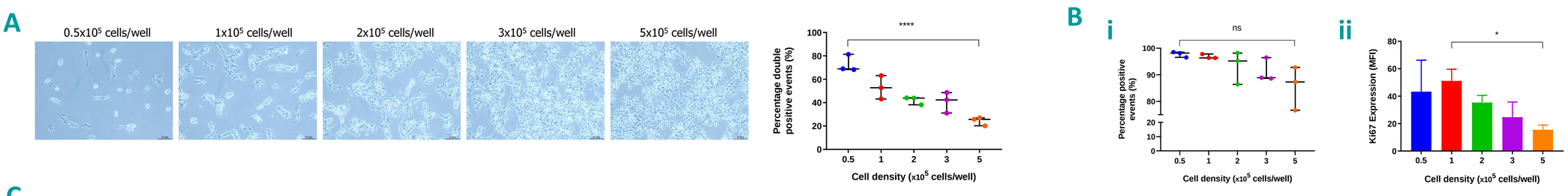



**Figure 2.** Expression of intracellular vimentin and citrullinated double positive events in B16F1 after stress induction. Significantly higher citrullinated vimentin after IFNγ treatment (a) and amino acid starvation (b) but not serum starvation (c). Expression of cell surface citrullinated vimentin and Annexin V as a marker for apoptosis in untreated and amino acid starved B16F1. Increased annexin V expression with amino acid starvation compared to untreated control. All citrullinated vimentin positive cells were apoptotic suggesting membrane disruption caused influx of calcium and subsequent citrullination.

## Higher citrullinated vimentin expression in autophagic, proliferative cells lacking cell-to-cell contact



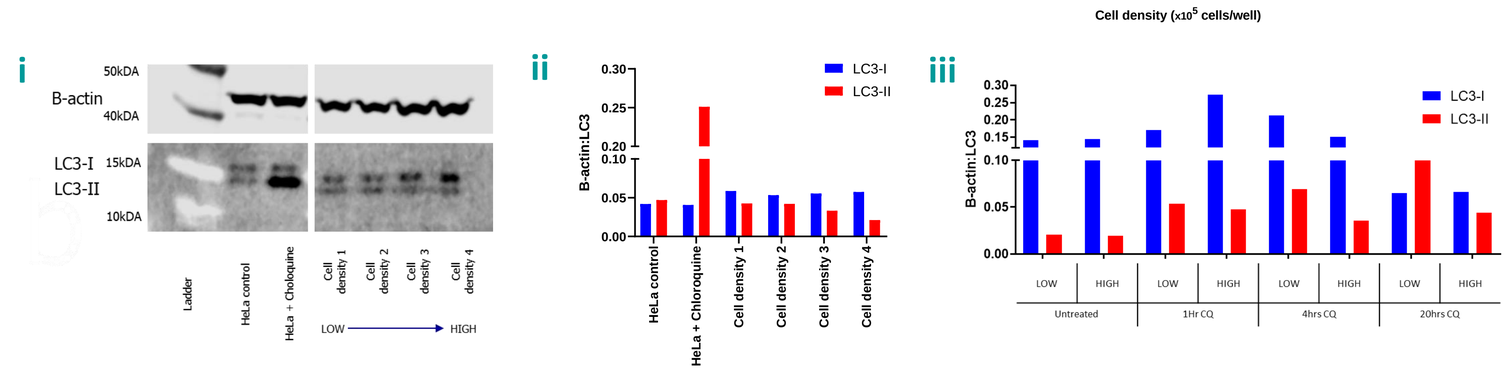




**Figure 3.** Expression of intracellular vimentin and citrullinated double positive events in B16F1 plated at 0.5, 1, 2, 3 and 5x10⁵ cells/well. Significantly greater expression at lower cell density (a). Proliferative marker Ki67 is only expressed during active cell cycle phases; G1, S, G2 and mitosis and absent during resting phase G0. Almost all cells expressed Ki67 in all densities (bi) but expression per cell was significantly reduced (bii). Expression of LC3-I and LC3-II in B16F1 plated at increasing cell densities via western blotting (ci). Intensity of LC3 bands were normalised against β-actin control (cii). No change in LC3-I but decrease in LC3-II (autophagosome bound molecule) at higher densities. Chloroquine treatment for 1, 4 and 20 hours show more autophagosome accumulation in low density compared to high density conditions (ciii).

## Repertoire of CD4 T cells in healthy individuals that respond to citrullinated peptides

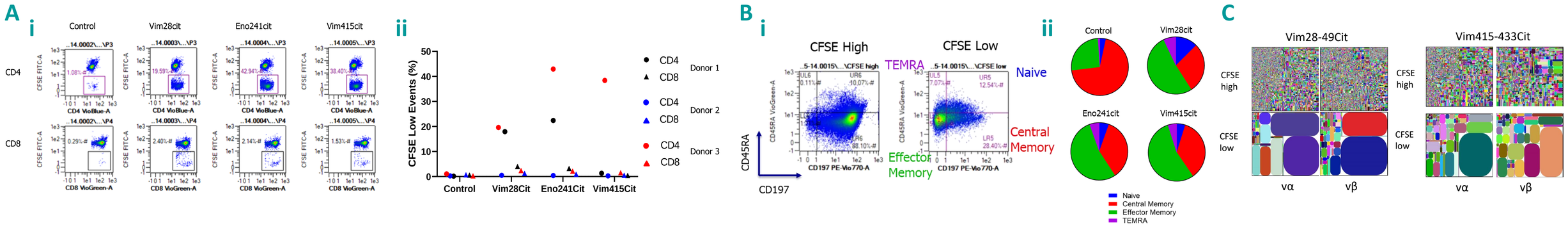



**Figure 4.** Proliferation of T cells (decrease in CFSE) in CD25 depleted PBMC population after incubation with citrullinated peptides for 10 days. Increase in CD4 T cells, not CD8, after exposure to citrullinated peptides; Vimentin28-49Cit, Enolase241-260Cit and Vimentin415-433Cit compared to vehicle control (ai-ii). Increase in T Effector Memory (CD45RA-ve, CD197+ve) and TEMRA population (CD45RA+ve, CD197-ve) in proliferating CD4 T cells after incubation with citrullinated peptides (bi-ii). CD4 cells were sorted on MoFlow sorter (Beckman Coulter) at day 10 and TCR repertoire analysed by iRepertoire Inc. Repertoire data for TCR Vα and Vβ shown as Tree plots where each spot denotes a specific V-J CDR3 and the spot size denotes frequency (c).

## CONCLUSIONS

- **IFNγ can induce citrullinated vimentin and MHC class II expression in tumour cells. Lack of expression after IFNγ treatment could be due to defects in CIITA.**
- **Nutrient deprivation significantly induced citrullinated vimentin expression but also showed high levels of apoptosis.**
- **Low density plated cells were the best inducers of citrullinated vimentin, the most proliferative and with the highest levels of autophagy.**
- **T cell responses in healthy human individuals to citrullinated vimentin peptides were mostly CD4 mediated and oligoclonal.**
- **Further phenotyping of the responding CD4 population found most cells were CD197(chemokine receptor 7; CCR7) negative suggesting they were Effector memory. Some had begun to re-express CD45RA; a marker mainly found on naïve T cells but this phenotype has also been described for a subset of highly cytotoxic T effector memory cells re-expressing CD45RA known as TEMRA [3].**

### References:

1. Brentville, V.A., et al., Citrullinated Vimentin Presented on MHC-II in Tumor Cells Is a Target for CD4+ T-Cell-Mediated Antitumor Immunity. Cancer Res, 2016. 76(3): p. 548-60.
2. Brentville, V.A., et al., T cell repertoire to citrullinated self-peptides in healthy humans is not confined to the HLA-DR SE alleles; Targeting of citrullinated self-peptides presented by HLA-DP4 for tumour therapy. OncoImmunology, 2019: p. 1-14.
3. Tian, Y., et al., Unique phenotypes and clonal expansions of human CD4 effector memory T cells re-expressing CD45RA. Nat Commun, 2017. 8(1): p. 1473.